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Physiological modulation of iron metabolism in rainbow trout (*Oncorhynchus mykiss*) fed low and high iron diets

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Summary

Iron (Fe) is an essential element, but Fe metabolism is poorly described in fish and the role of ferrireductase and transferrin in iron regulation by teleosts is unknown. The aim of the present study was to provide an overview of the strategy for Fe handling in rainbow trout, *Oncorhynchus mykiss*. Fish were fed Fe-deficient, normal and high-Fe diets (33, 175, 1975 mg Fe kg^{-1} food, respectively) for 8 weeks. Diets were chosen so that no changes in growth, food conversion ratio, haematology, or significant oxidative stress (TBARS) were observed. Elevation of dietary Fe caused Fe accumulation particularly in the stomach, intestine, liver and blood. The increase in total serum Fe from 10 to 49 μmol l^{-1} over 8 weeks was associated with elevated total Fe binding capacity and decreased unsaturated Fe binding capacity, so that in fish fed a high-Fe diet transferrin saturation increased from 15% at the start of the experiment to 37%. Fish on the high-Fe diet increased Fe accumulation in the liver, which was correlated with elevation of hepatic ferrireductase activity and serum transferrin saturation. Conversely, fish on the low-Fe diet did not show tissue Fe depletion compared with normal diet controls and did not change Fe binding to serum transferrin. Instead, these fish doubled intestinal ferrireductase activity which may have contributed to the maintenance of tissue Fe status. The absence of clear treatment-dependent changes in branchial Fe accumulation and ferrireductase activity indicated that the gills do not have a major role in Fe metabolism. Some transient changes in Cu, Zn and Mn status of tissues occurred.

Key words: rainbow trout, *Oncorhynchus mykiss*, dietary iron, transferrin, ferrireductase, intestine.

Introduction

Iron is an essential element that has a number of fundamental roles in cellular biochemistry and metabolism. These include oxygen binding to heme proteins and the formation of active centres in enzymes involved in the mitochondrial electron transport chain (De Silva et al., 1996; Aisen et al., 2001). Iron can also vary its redox state and can be rapidly oxidised from Fe^{2+} to Fe^{3+} (ferrous to ferric iron) in the presence of oxygen. This reaction generates the superoxide anion, which through a series of redox reactions leads to the generation of toxic hydroxyl radicals (the Haber-Weiss reactions; De Silva et al., 1996; Aisen et al., 2001). Thus iron can be both toxic and beneficial to organisms, and iron status in the body must be carefully regulated to provide sufficient iron for biological functions, whilst avoiding excess Fe^{2+} which can lead to oxidative stress.

Fish acquire iron predominantly from the diet, and with negligible iron uptake at the gills compared with the gut (Andersen, 1997; Bury et al., 2001), teleost fish have a dietary iron requirement of ~30–200 mg kg^{-1} dry mass (d.m.) of food (for reviews, see Davis and Gatlin, 1991; Watanabe et al., 1997). There are only a few reports of dietary iron deficiency in fish, and these have focused on defining the nutritional requirements to avoid anaemia and growth retardation in aquaculture (Kawatsu, 1972; Sakamoto and Yone, 1978; Davis and Gatlin, 1991; Watanabe et al., 1997). A precise iron requirement for most fish species, including rainbow trout, remains to be determined. However, normal dietary levels of ~100–250 mg Fe kg^{-1} d.m. food have been suggested for salmonids (Desjardins et al., 1987; Andersen et al., 1996). A few studies have used large excesses of dietary iron to explore the role of iron in oxidative stress in fish, as indicated by lipid peroxidation products in the liver (e.g. 6.3 g Fe kg^{-1} d.m. of food, African catfish; Baker et al., 1997). Despite this information on the nutritional requirement and toxic effects of iron, few attempts have been made to explore physiological regulation and mechanisms of iron metabolism in fish. However, two early studies using injected ^{59}Fe suggest the liver is the main storage pool for iron in fish (in tench, *Tinca tinca* L.; Van Dijk et al., 1975; rainbow trout, *Oncorhynchus mykiss*; Walker and Fromm, 1976).

Iron forms insoluble ferric (hydro)oxides at neutral pH (Aisen et al., 2001) and molecular evidence suggests that the

small fraction of Fe^{3+} presumably present in the gut lumen will be reduced to Fe^{2+} prior to import into the gut enterocytes of fish (Bury et al., 2003). In mammals, ferrireductase activity in the brush border of the intestinal mucosa facilitates the reduction of Fe^{3+} to Fe^{2+} (Riedel et al., 1995; McKie et al., 2001) prior to Fe^{2+} import on divalent metal ion transporter 1 (DMT 1, Gunshin et al., 1997; Trinder et al., 2000). Although intestinal ferric reductase activity has not been measured in rainbow trout, in the European flounder at least (Bury et al., 2001), Fe^{2+} is absorbed three times faster than Fe^{3+}. DMT 1 genes are also expressed in fish intestine, for example, rainbow trout (Dorschner and Phillips, 1999) and zebrafish (Donovan et al., 2002).

Intracellular Fe is stored as Fe^{3+} by ferritin, a 450 kDa protein with a spherical cavity capable of carrying 4500 iron atoms (Aisen et al., 2001). Ferritins are an ancient group of proteins conserved in bacteria, plants and man (Aisen et al., 2001), and have also been found in fish (Andersen, 1997). The precise mechanism of how imported Fe^{2+} is re-oxidised to Fe^{3+} by cytoplasmic ferritin, or how the Fe^{3+} is subsequently reduced to Fe^{2+} for export from the cell to the blood, remains controversial in mammals (Reilly and Aust, 1998; Winzerling and Law, 1997; Aisen et al., 2001) and unknown in fish. In mammals basolateral export of Fe^{2+} from the cell to the blood is against the electrochemical gradient, and probably mediated by iron regulated transporter (IREG 1, also called MTP 1 or ferroportin), and recent evidence from the zebrafish genome suggests IREG 1 genes are present in fish (Donovan et al., 2000; Bury et al., 2003). In mammals, exported Fe^{2+} is oxidised on the extracellular surface of the serosal membrane by a membrane bound copper oxidase (a ceruloplasmin homologue, hephaestin), and the resulting Fe^{3+} binds rapidly to extracellular transferrin to facilitate bulk iron transport in the blood (Winzerling and Law, 1997; Aisen et al., 2001). Circumstantial evidence argues for a similar iron export process in fish cells (Bury et al., 2003). Fish have long been known to have transferrin for bulk iron transport in the blood (Hershberger, 1970; Ikeda et al., 1972), and in the hagfish (*Myxine glutinosa*) at least, the transferrin has a similar structure to that in humans (Aisen and Leibman, 1972).

In this paper, we give a detailed account of dietary iron accumulation and distribution in rainbow trout, and for the first time demonstrate that rainbow trout modulate ferrireductase activity in the intestine, the pool of transferrin in the blood and Fe storage in the liver, to control whole body iron status. We also present details of tissue Cu, Zn and Mn levels in relation to iron status because of apparent promiscuity of the iron import mechanisms (DMT 1; Gunshin et al., 1997) and the involvement of Cu-dependent oxidases in facilitating iron transport across cell membranes (Winzerling and Law, 1997).

Materials and methods

Stock animals

Rainbow trout, *Oncorhynchus mykiss* Walbaum 1792, weighing 79.1±12.2 g (mean ± S.E.M., from a sample of 31 stock fish) were obtained from a local fish farm (Hatchland Fisheries, Rattery, Devon) and held for at least 14 days in stock aquaria supplied with recirculating, aerated and dechlorinated Plymouth tap water (see below). Stock fish were fed daily to satiation on a commercial trout food [Trouw Aquaculture, trout feed No. 3, containing 28.2% wheat, 20.0% soya bean extract, 13.0% animal protein, 5.5% fat/molasses, 3.3% vitamin/mineral supplement; % d.m. of feed (manufacturer's information)].

Experimental design

Fish were randomly allocated to one of three dietary iron treatments (see below) containing no added iron, a normal level of iron (+100 mg Fe kg^{-1} dry mass; d.m.), or an elevated level of iron (+1500 mg Fe kg^{-1} d.m.), for up to 8 weeks. These iron concentrations and treatment times were chosen to generate total iron doses that would stimulate a physiological response, whilst avoiding toxic effects such as reduced growth and anaemia. Each treatment was performed in triplicate (three tanks/treatment, nine tanks in all) with 44 fish/tank (132 fish/treatment). Fish were starved for 48 h to clear the gut contents before starting the experiment, and were then fed twice daily on one of the iron treatments for 8 weeks, using a measured food ration of 1.5% body mass per day. Fish biomass per tank was measured fortnightly so that the ration could be maintained at 1.5% throughout the experiment. All food was eaten, and faecal waste from the fish was flushed by the self-cleaning design of the tanks (99% replacement of water in 12 h) and routine siphoning after feeding. Nonetheless, water quality was monitored before and after feeding and aqueous iron levels remained at or below the detection limit. The water quality was identical to stock conditions (mean ± S.E.M., N=8, in mmol l^{-1}), Na, 0.36±0.01; K, 0.17±0.02; Ca, 0.41±0.01; Cu, <0.0001; Zn, 0.0010±0.0001; Fe, <0.0005 at pH 7.7±0.07 and 15±1°C. Fish were collected (before the daily feeding) at fortnightly intervals for tissue biochemistry, haematology, nutritional performance and trace metal composition (see below).

Diet formulation

Diets were formulated using purified casein (Natural Adhesive Co., Daventry, UK) that was low in iron compared with fish meal-derived protein sources, and thus enabled modification of the iron content of the diet by the addition of $FeSO_4.7H_2O$. The formulation was (in g kg^{-1} d.m. of food) casein, 434.6; corn starch, 205.5; cod liver oil, 18; mineral premix, 100; fish meal, 50; vitamin premix, 20; carboxymethyl cellulose, 10; astaxanthin, 0.5. Measured proximate composition of the diet was (% dry matter) 41% protein, 17% lipid and 14% ash. The vitamin premix (Trouw, standard salmonid premix) contained ascorbyl polyphosphate (100 mg kg^{-1} food; Rovimix StayC-vit, Ròche Ltd, Basle, Switzerland) to prevent auto-oxidation of the food. The mineral premix was formula MIN-101 of Cho et al. (1985). Diets were prepared by mixing 1 kg of dry ingredients with either zero, 0.49 or 7.44 g of $FeSO_4.7H_2O$ powder to give

nominal added iron premix to the food of 0, 100 and 1500 mg Fe kg^{-1} dry matter. Measured Fe contents of diets were (mean ± S.E.M., N=5) 33.0±1.5, 174.5±9.5 and 1974.8±145 mg Fe kg^{-1} dry matter and hereafter are called low-, normal- and high-iron diets respectively. The low-Fe diet was the lowest Fe content of food possible based on available feed ingredients, whilst the normal diet was selected to be in the normal range suggested for salmonids, of between 100–250 mg Fe kg^{-1} dry matter (Andersen et al., 1996; Desjardins et al., 1987). The high-Fe diet was selected to be above the normal range, but below doses known to cause overtly depressed growth and toxicity in fish (Baker et al., 1997).

Growth and nutritional performance

Specific growth rate (SGR, % gain in body mass per day) was calculated from the mean weights of fish at time zero and week 8 for each treatment. Food conversion ratio (FCR) was calculated from the food intake per fish (1.5% ration) divided by the mean body weight gain of fish in each treatment over 8 weeks. Condition factor (K=100 × mass/standard length^3) was calculated from fish weights at the end of the experiment. The proximate composition of the whole body (including the washed gut) was determined (Baker and Davies, 1996) for seven fish taken at random from the tanks at time zero (initial fish) and for a further seven fish per treatment at week 8.

Haematology and trace metal analysis

Fish (nine/treatment, three from each tank) were anaesthetised with benzocaine, weighed and standard length recorded. Blood was collected by caudal pucture into a non-heparinised syringe and divided into heparinised (for haematology) and non-heparinised (for iron assays) Eppendorf tubes. Haematology was performed immediately (Handy and Depledge, 1999) and an aliquot of plasma collected for Cu analysis (Handy et al., 1999). Non-heparinised blood samples were allowed to clot for 2–3 h in a refrigerator before iron assays. Total iron in the serum (TI), unsaturated iron-binding capacity (UIBC) and total iron-binding capacity (TIBC) were measured using a diagnostic kit (Sigma Diagnostics, kit N°. 565, manual procedure). The iron assays were adapted for microplates by using 50 µl of serum in 250 µl of iron buffer reagent plus 5 µl of colour reagent

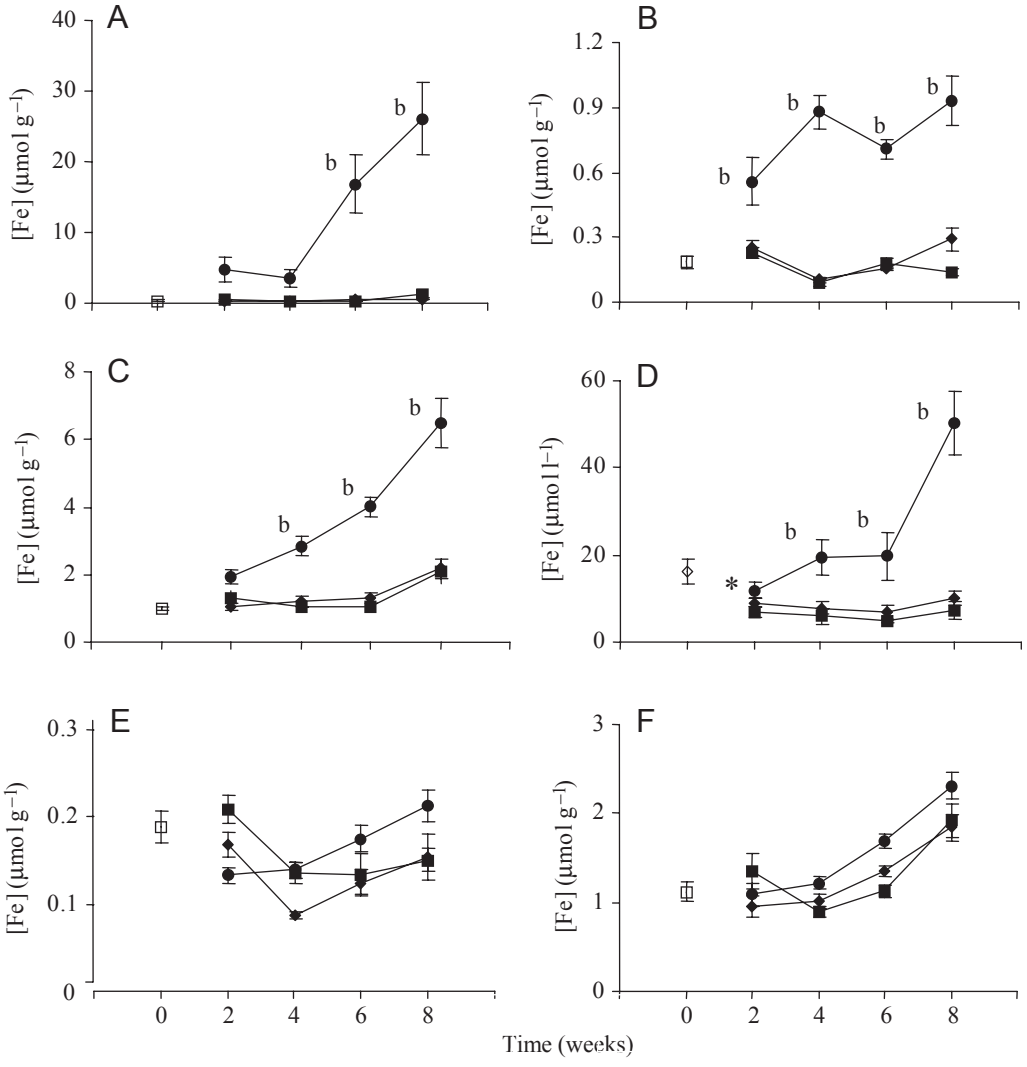

Fig. 1. Effect of casein-based diet containing low (filled square), normal (filled diamond) and high (filled circle) levels of dietary Fe for 8 weeks (measured Fe in diet, 33, 175 and 1975 mg kg^{-1}, respectively) on tissue Fe concentration in the intestine (A), stomach (B), liver (C), serum (D), muscle (E) and gill (F) of rainbow trout. Values are means ± S.E.M. (N=9 fish per time point). Different letters (a, b or c) denote statistically significant differences in tissue Fe concentration within each time point between experimental dietary Fe treatments (ANOVA followed by LSD test, P<0.05). All data are denoted the letter a (not shown for clarity in figures) unless otherwise indicated. Thus, b indicates the high-Fe diet is different from either low or normal diets in the panels for Fig. 1, but low and normal diets are not different from each other. No treatment differences were observed in muscle or gill. Asterisk indicates a significant difference between initial values (open symbol) in stock fish fed normal commercial trout food compared with fish at week 2 on the normal Fe diet (Student's t-test, P<0.05, N=9).

(total serum iron assay), and 50 µl serum with 200 µl of UIBC buffer and 50 µl standard plus 5 µl colour reagent (UIBC assay). The absorbance of samples was read at 550 nm on a Dynex MRX plate reader. TIBC was calculated from total iron plus UIBC. The percentage of transferrin saturated with Fe was calculated as (100-(UBIC/TIBC))×100%, according to the Sigma protocol.

Fish were immediately dissected after blood sampling to collect gill, liver, stomach, intestine (minus pyloric caecae) and muscle for metal analysis according to the method of Handy et al. (1999) with modifications (Handy et al., 2000). Briefly, tissues were digested in 5 ml of concentrated nitric acid at 60°C for 4 h, diluted to 20 ml and analysed by inductively coupled plasma atomic emission spectrophotometry (ICP-AES; Varian Liberty 200). Whole body Fe concentration was also measured in dry ground carcass prepared for proximate composition. Working detection limits of matrix matched standards were (in µmol l^{-1}): Na, 0.27, Fe, 0.04; Zn, 0.01; Cu, 0.03; K, 0.27; Mn, 0.01. Serum samples were also analysed for Cu (100 µl diluted to 1.5 ml in deionised water). Serum chloride was determined by automated titration (Corning Chloride Meter 920).

Biochemistry and histology

A further seven to nine fish per treatment were collected every 2 weeks for biochemistry. Fish were anaesthetised and weight/length measured as above. Whole gill filaments, liver and intestine (posterior to the pyloric caecae) were carefully dissected, rinsed in distilled water and snap frozen in liquid nitrogen, then stored at –80°C for subsequent analysis. At weeks 0, 4 and 8 samples of these tissues were also fixed in buffered formal saline, prepared for routine wax histology, and stained with Mallory's trichrome (Handy et al., 1999).

Tissue homogenates for biochemistry were prepared by adding 0.5 g of defrosted tissue to 2 ml of ice cold Dulbecco's phosphate-buffered saline (PBS, pH 7.4, + 1 mmol l^{-1} EDTA) and homogenised (Ultra Turrax T8, IKA Labortechnik, 4 mm dia. shaft, 2×15 s bursts with a 30 s rest in ice, maximum speed). Homogenates were aliquoted into Eppendorf tubes and frozen at –80°C until required for protein determination (Handy and Depledge, 1999), thiobarbituric acid reactive substances (TBARS; Camejo et al., 1998), and NADH-dependent ferrireductase activity (Schulte and Weiss, 1995).

Calculations and statistics

Data were analysed on Statgraphics Plus for Windows version 4.0 or Statistica version 6.0. as described by Handy et al. (1999) using 2-way ANOVA with LSD multiple range test. The ANOVA used dietary Fe treatment as the first factor, and time as the second factor, to compare the effects of the experimental diets from weeks 2 to 8. Non-parametric data were transformed (log or square root) as appropriate prior to ANOVA and the LSD test. Comparisons between initial fish on commercial trout food and the normal diet controls on the casein-based diet were made using the Student's *t*-test. A rejection level of P=0.05 was used for all analysis. The percentage distribution of iron in organs was calculated from the ratio of absolute metal content of organ:whole body. Absolute metal content for the whole body was calculated from whole body metal concentration multiplied by fish weight. Absolute metal content of organs was calculated from whole organ weights (derived from Barron et al., 1987; except liver which was directly measured) multiplied by metal concentration of the tissue. For blood, absolute Fe content was calculated assuming a plasma volume of 2.27 ml 100 g^{-1} (Gingerich and Pityer, 1989). Apparent net retention of metals was calculated from cumulative food intake and absolute change in metal content of the whole body during the experiment (Baker et al., 1998).

Results

Iron accumulation and distribution

Elevation of dietary Fe above normal caused Fe accumulation in the fish, but the Fe-deficient diet generally did not cause tissue Fe depletion (Fig. 1; Table 1). Fish fed the high-Fe diet showed statistically significant increases in Fe concentration in the intestine, stomach, liver and serum, but not muscle or gill compared with fish on either normal or low-Fe diets (diet effect ANOVA, P<0.05). The biggest increases occurred in the intestine (86-fold increase), liver

Table 1. *The effect of dietary iron on the percentage of whole body iron in each organ after 8 weeks*

Dietary Fe	Gill	Intestine	Stomach	Liver	Muscle	Serum
Low	16.3±1.1[a]	5.6±1.2[a]	1.0±0.1[a]	9.6±1.1[a]	42.6±2.0[a]	0.07±0.01[a]
Normal	17.3±2.0[a]	2.8±0.5[b]	2.0±0.2[b]	10.5±1.3[a]	42.6±2.9[a]	0.11±0.01[b]
High	6.4±0.9[b]	37.7±4.0[c]	2.0±0.3[b]	9.7±1.1[a]	19.2±2.2[b]	0.15±0.02[c]

Values are percentages of whole body iron (Fe) (mean ± S.E.M., N=9/treatment) at the end of the experiment. Other details and statistics as in Fig. 1. Data were calculated from the absolute ratio of absolute Fe content in each organ and the whole fish (see Materials and methods for details). Whole body Fe concentrations at week 8 were (in µmol g^{-1} d.m.; mean ± S.E.M. N=9 per treatment): 0.24±0.02, low-Fe diet; 0.23±0.03, normal-Fe diet; 0.87±0.09 high-Fe diet (fish on high-Fe diet were statistically different from those on other diets, Student's *t*-test, P<0.05). Whole body Fe concentration in initial fish was 0.22±0.02 µmol g^{-1} d.m. (mean ± S.E.M., N=9). % Fe distribution in the organs of initial fish were (mean ± S.E.M., N=9): 10.2±1.03, gill; 1.9±0.1, intestine; 1.4±0.2, stomach; 5.5±0.3, liver; 56.0±1.4, muscle; 0.18±0.04, serum.

Different letters indicate statistically different values within columns (Student's *t*-test, P<0.05).

Table 2. *Haematological parameters in rainbow trout after 8 weeks on low-, normal- or high-iron diets*

Dietary Fe	Hb (g dl^{-1})	Hct (%)	RBC count (10^6 cells mm^{-3})	MEV (nm^3)	MEH (mg cell^{-1})
Low	6.6±0.21^a	42.3±2.2	1.16±0.04	366±21	57.3±2.1^a
Normal	7.8±0.22^b	43.3±2.3	1.15±0.04	394±19	68.9±2.3^b
High	7.9±0.15^b	37.9±1.1	1.13±0.04	338±14	70.2±2.0^b

Data are means ± S.E.M. (N=9 fish per treatment). Hb, haemoglobin; Hct, haematocrit; RBC count, red blood cell count; MEV, mean erythrocyte volume; MEH, mean erythrocyte haemoglobin content. Other details and statistics as in Fig. 1. No treatment-related effects were observed in HCT, RBC or MEV. Values for initial fish were (mean ± S.E.M., N=9): Hb, 7.8±1.0; Hct, 35.3±2.3; RBC count, 1.40±0.18; MEV, 255±30; MEH, 55.9±8.0.

Different letters indicate statistically different values within columns (Student's t-test, P<0.05).

(6.5-fold increase) and stomachs (3.9-fold increase) of fish fed the high-Fe diet, compared with the initial fish (Fig. 1). These differences were apparent as early as week 2 of the experiment for Fe levels in the stomach, week 4 for Fe levels in the liver and serum, and week 6 for Fe levels in the intestine on the high-Fe treatment compared with normal or low-Fe diets. These time-effects in tissues persisted until the end of the experiment (time effect, ANOVA, P<0.05). Gill tissue did not show any treatment-dependent change in Fe at each time point (Student's t-test, P>0.05), but the ANOVA indicated the rate of elevation of Fe in the gills of fish on the high-Fe diet was faster than either control or Fe-deficient treatments over the entire experiment (time × dietary Fe interaction, ANOVA, P<0.05). The high-Fe group showed a 1.3-fold increase in gill Fe compared with a 0.7-fold increase in the gills of the low-Fe group over the entire experiment (Fig. 1).

Fish on the low-Fe diet, were able to maintain whole body Fe levels comparable with those of controls, but fish on the high-Fe diet showed a 3.7-fold increase in whole body Fe compared with controls (Student's t-test, P>0.05). This was reflected by an apparent redistribution of body Fe to the serum and intestine of fish fed the high-Fe diet (Table 1). The intestine of the high-Fe fed group supporting 37% of the body burden by the end of the experiment compared with only 1% at the start (Table 1). Similarly, the percentage of body burden in the serum increased 15 fold over the entire experiment. The proportion of whole body Fe stored in the gills of fish fed the high-Fe diets were about 10% lower than either the control or low-Fe diet treatments. However, the low-Fe diet did not significantly reduce the proportion of whole body Fe stored in the serum or intestine. The percentage body burden of Fe in the liver remained constant at about 10% of the whole body Fe for all treatments (Table 1).

Iron binding in the blood

Dietary Fe treatment did not affect Hct or MEV, and there was only a small (but statistically significant, ANOVA and LSD, P<0.05) decrease in red cell haemoglobin content in the low-Fe fed fish compared with the other treatments at the end of the experiment (Table 2). Serum Cl^- remained in the normal range throughout the experiment (in mmol l^{-1}; mean ± S.E.M., N=27 fish per treatment; low Fe, 113±3; normal Fe, 114±3; high Fe, 114±2). However, there were major changes in the

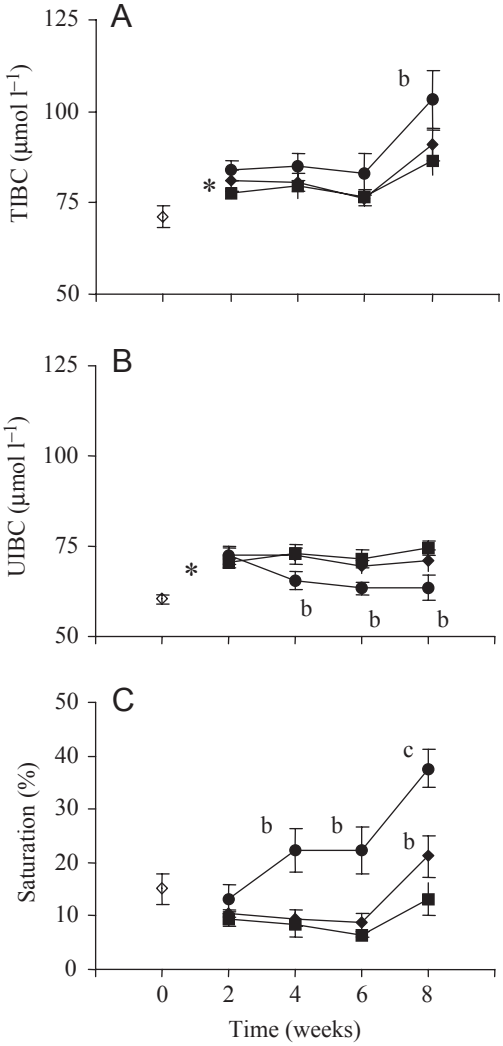

Fig. 2. (A) Total iron binding capacity (TIBC), (B) unsaturated iron binding capacity (UIBC) and (C) the percentage of transferrin in the serum saturated with Fe in rainbow trout fed casein-based diets containing low (filled square), normal (filled diamond) and high (filled circle) levels of Fe for 8 weeks. Values are means ± S.E.M. (N=6). Other details and statistics as in Fig. 1.

iron storage pattern of the serum (Fig. 2). Elevation of total serum Fe concentration in response to the high-Fe diet (Fig. 1) was reflected in an increase in the TIBC of the serum, and a simultaneous decrease in UIBC, leading to a 1.5-fold increase in the saturation of transferrin in the serum (Fig. 2). At the end of the experiment, fish on the high-Fe diet showed a 37% saturation of the transferrin pool in the blood, compared with only 15% in the initial fish at the start of the experiment (Fig. 2). The Fe binding characteristics of the serum in fish fed the low-Fe diet were not different from fish on the normal-Fe diet, except at the end of the experiment where saturation of transferrin decreased compared with that in fish fed the normal diet (Fig. 2).

Although treatment-dependent effects on total serum Fe (Fig. 1), and saturation of transferrin in the serum occurred from week 4 onwards (Fig. 2), there were also some effects of switching from the commercial trout food to the casein-based experimental diets. Serum total Fe concentration in the initial fish at the start of the experiment was higher than in all treatments at week 2 (Student's *t*-test, $P<0.05$). Furthermore, TIBC and UIBC were significantly lower (Student's *t*-test, $P<0.05$) in the initial fish compared with fish fed the normal-Fe experimental diet (Fig. 2) and also the high- or low-Fe diets, suggesting that the casein diet stimulates the appearance of new (unsaturated) transferrin in the blood to increase the UIBC relative to that in the initial fish.

NADH-dependent ferrireductase activity

NADH-dependent ferrireductase activity was measured in gill, intestine and liver (Fig. 3). NADH-dependent ferrireductase activity was significantly affected by the dietary Fe treatments (ANOVA, $P>0.05$) in liver and intestine. There was a marked and progressive stimulation of ferrireductase activity in the intestine of fish fed the low-Fe diet over the experiment (ANOVA, $P<0.05$). Conversely, by week 8 the high-Fe diet caused a significant increase (ANOVA and LSD, $P<0.05$) in ferrireductase in the liver compared with the other treatments (Fig. 3). Switching from the commercial trout food to the casein based diet (initial fish compared with controls at week 2) had no effect on enzyme activities (Student's *t*-test, $P>0.05$).

Ferrireductase activity was also correlated with tissue Fe concentration. In the intestine, ferrireductase activity was inversely related to the log of tissue Fe concentration, with fish fed either the high or low-Fe diets fitting on the same trend line. Interestingly, values from fish on the normal-Fe diet clustered together away from the trend line suggesting that there may be endogenous and inducible forms of intestinal ferrireductase (Fig. 3B inset). Ferrireductase activity in the liver showed the opposite trend to the intestine, with hepatic ferrireductase increasing with the log of liver Fe concentration,

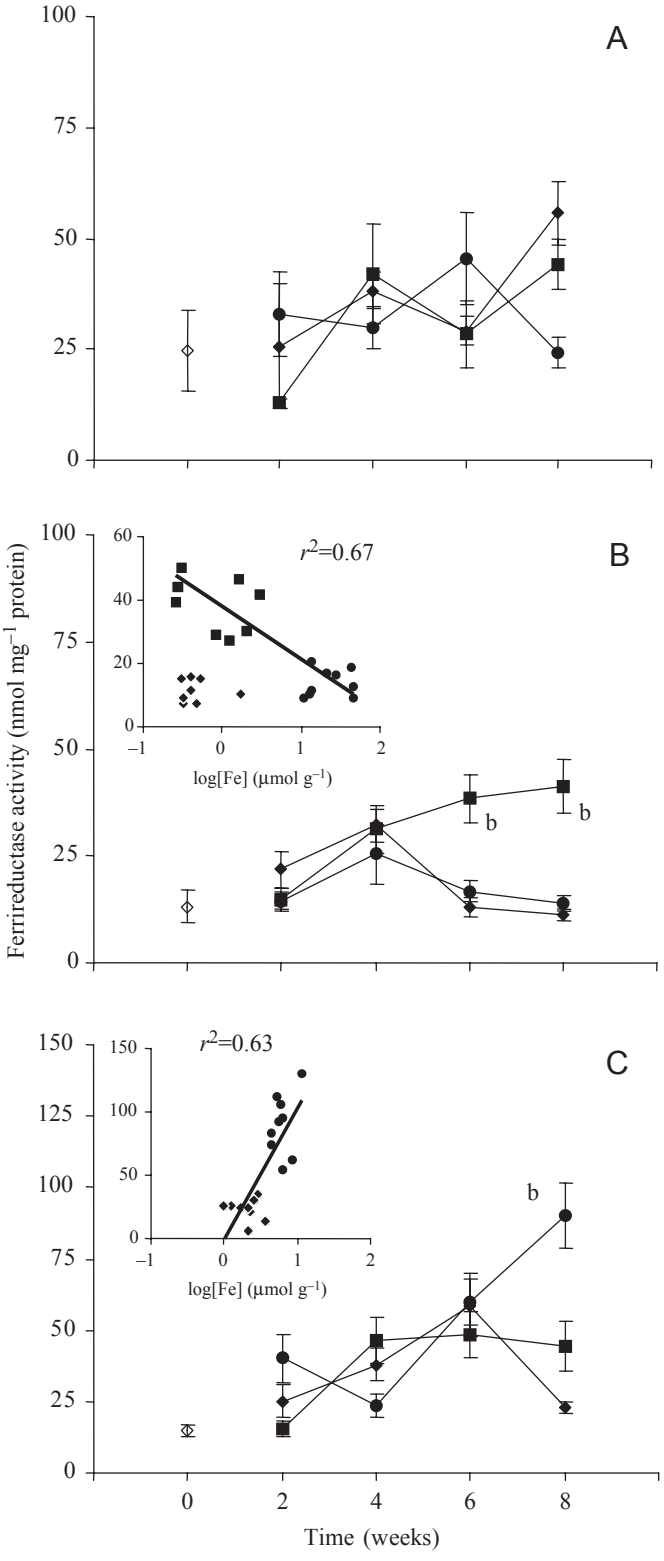

Fig. 3. NADH-dependent ferrireductase activity in gill (A), intestine (B) and liver (C) of rainbow trout fed a casein-based diet containing low (filled square), normal (filled diamond) and high (filled circle) levels of Fe for 8 weeks. Values are means ± S.E.M. (N=6). Other details and statistics as in Fig. 1. No differences were observed between treatments in gill tissue. No differences were observed between initial fish and those on the normal Fe diet at week 2. Insets are log[Fe] in tissue correlated with ferrireductase activity for intestine (B inset, y=–16.63x+38.10 for low- and high-Fe diets, N=18) and liver (C inset, y=105.03x–1.24, for normal and high-Fe diets, N=18). Note, ferrireductase activity is normalised per minute (nmol mg^{-1} min^{-1}).

but only in fish on the normal and high-Fe diets (Fig. 3C inset). Hepatic Fe concentration and ferrireductase activity were also correlated with the percentage saturation of transferrin in the serum (Fig. 4), indicating that increased Fe loading of the liver in response to Fe overload in the blood is partly facilitated by increased ferrireductase activity. Hepatic ferrireductase did not correlate with liver Fe concentration or serum saturation in fish from the low-Fe diet (data not shown).

Thiobarbituric acid reactive substances (TBARS)

TBARS broadly indicates the level of oxidative stress in the tissue (Fig. 5). There was a significant effect of dietary Fe on TBARS in the intestine and liver (ANOVA, $P<0.05$), but no effect on the gills. Notably, TBARS in the intestine of fish fed the high-Fe diet was significantly elevated by week 4 and remained above normal for the entire experiment (Fig. 5B). Hepatic TBARS were also elevated in the high-Fe fed fish by week 8. TBARS in the intestine were also linearly related to the log of intestinal Fe concentration for all fish (inset, Fig. 5B), and similarly in the liver of fish on normal and high Fe-diets (inset, Fig. 5B). Switching from the commercial food to the casein-based diet (initial fish compared with 2 week controls) had no statistically significant effect (Student's *t*-test, $P>0.1$).

Nutritional performance

The different dietary iron treatments had no effect on growth, FCR, or proximate composition of the fish (Table 3). However, faecal Fe concentration increased significantly with dietary Fe intake (Student's *t*-test, $P<0.001$ for all comparisons) and this was also reflected in a fall in apparent net Fe retention with increasing dietary Fe concentration (Table 3).

Histology

There were no pathological changes in the gross anatomy of the gills, intestine or liver associated with the dietary iron treatments by the end of the experiment (week 8). The gills of all fish were normal (not shown) with no signs of oedema or mucocyte proliferation. The intestinal mucosa of fish were also intact and healthy. However, the mucous epithelium of the fish fed the low-Fe diet had fewer goblet cells, and enterocytes contained fewer absorptive vacuoles, than the intestines of fish from either the normal or high-Fe diet (not shown). The livers of all fish were also normal and without overt pathology, although there were some subtle changes in intracellular glycogen storage and sinusoid space between treatments (see Fig. 6 for details).

Trace element composition

Fish tissues were also analysed for Cu, Zn and Mn, in addition Na and K were also measured in the gills and intestines. Dietary Fe status had no effect on gill or intestine Na concentration, and the intestine from fish on the low Fe-diet showed a persistent trend of K depletion (not statistically significant, data not shown).

Dietary Fe had no clear treatment-dependent effect on tissue Cu levels that persisted over time during the experiment. However, there were some transient changes in Cu in some tissues. In the early stages of the experiment (weeks 2), fish fed the low-Fe diet had a significantly higher muscle Cu concentration (ANOVA and LSD, $P<0.05$) compared with either normal- or high-Fe treatments (Cu in μmol g^{-1} d.m.; mean ± S.E.M., $N=9$; low Fe, 0.023±0.001; normal Fe, 0.015±0.002; high Fe, 0.016±0.002). This treatment difference in muscle Cu was absent by week 6. At the end of the experiment Cu concentrations in the intestine of fish fed the low-Fe diet were significantly higher (ANOVA and LSD, $P<0.05$) that the other treatments (Cu in μmol g^{-1} d.m., mean ± S.E., $N=9$; low Fe, 0.521±0.067; normal Fe, 0.299±0.094; high Fe, 0.194±0.035). A correlation analysis of intestinal Fe *versus* Cu concentration (regardless of treatment) at week 8 yielded a negative correlation (correlation coefficient=–0.27, $P<0.05$), indicating that reductions in intestinal tissue Fe levels was partly responsible for increases in intestinal Cu concentration. No notable treatment effects of dietary Fe on tissue Cu status were apparent for stomach, gill or liver (data not shown).

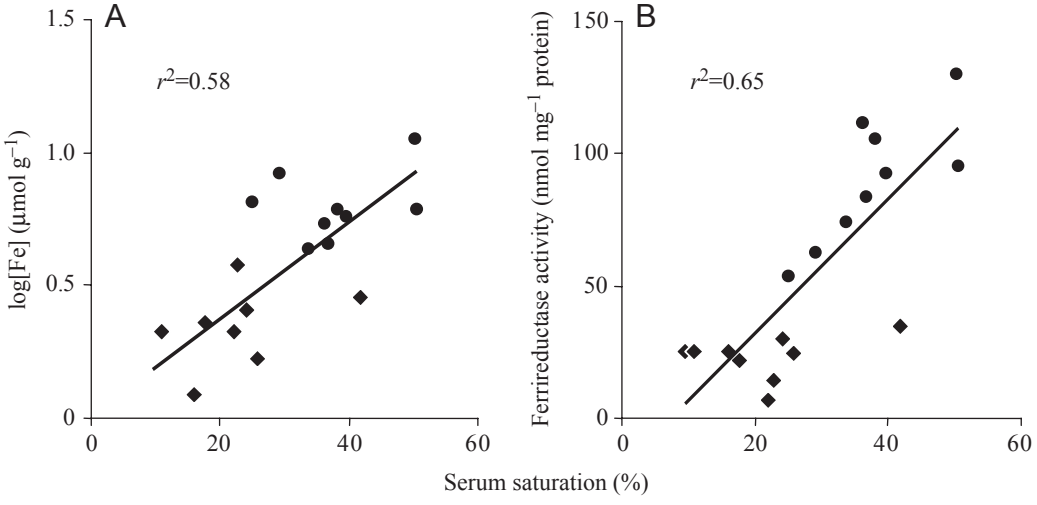

Fig. 4. Correlation between percentage Fe saturation of transferrin in the serum and log[Fe] in the liver (A), and liver ferrireductase activity (B) in rainbow trout fed a casein-based diet containing normal (filled diamond) and high (filled circle) levels of iron. The equation for the linear fits are $y=0.02x+0.01$ and $y=2.55x-18.56$ for A and B, respectively ($N=18$). Note, ferrireductase activity is normalised per minute (nmol mg^{-1} min^{-1}).

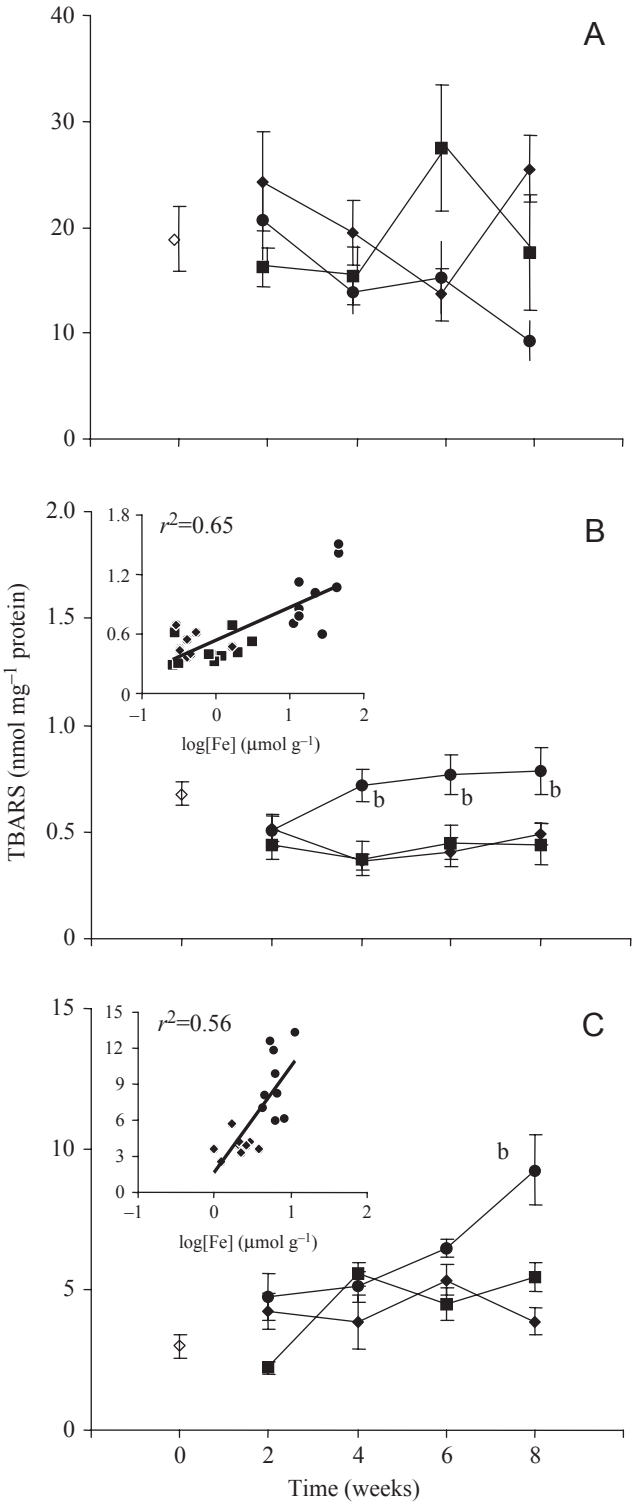

Fig. 5. Thiobarbituric acid reactive substances (TBARS) in gill (A), intestine (B) and liver (C) of rainbow trout fed a casein-based diet containing low (filled square), normal (filled diamond) and high (filled circle) levels of iron. Values are means ± S.E.M. (N=6). Other details and statistics as in Fig. 1. No statistical differences were observed between TBARS of initial fish and those on the normal-Fe diet at week 2. Insets are log[Fe] in tissue correlated with TBARS for intestine (B inset, y=0.33x+0.55, for all diets N=27) and liver (panel C insert, y=8.79x+1.72, for normal and high-Fe diets, N=18).

Dietary Fe alone had no effects on the Zn status of the stomach, intestine, liver or gill of the fish (data not shown). There was, however, a statistically significant transient rise in Zn concentration in the muscle of fish fed the low-Fe diet at week 4 compared with the other treatments (Zn in μmol g^{-1} d.m.; mean ± S.E.M., N=9; low Fe, 0.284±0.017; normal Fe, 0.155±0.008; high Fe, 0.195±0.001).

Dietary iron level was generally inversely related to tissue Mn, indicating that elevation of dietary Fe caused tissue Mn depletion, and reduction of dietary Fe caused Mn overload. This effect was most notable (ANOVA and LSD, P<0.05) in the intestine at week 2 (Mn in μmol g^{-1} d.m., mean ± S.E.M.,

Table 3. *Nutritional performance of rainbow trout after 8 weeks on low-, normal- or high-iron diets*

Variable	Dietary Fe		
	Low	Normal	High
Initial mean mass (g)		79.1±5.0	
Final mean mass (g)	124.2±7.8	133.5±12.1	130.7±12.1
Index			
SGR (% day)	0.92±0.13	1.04±0.19	0.92±0.22
FCR	1.95±0.5	2.03±0.71	1.97±0.49
Condition factor	1.18±0.05	1.20±0.05	1.20±0.05
Hepatosomatic index (%)	1.14±0.09	1.11±0.13	1.15±0.08
Fe retention			
Faecal [Fe] (μmol g^{-1} d.m.)	3.4±1.1[a]	12.3±3.5[b]	45.1±2.9[c]
Final whole body Fe content (μmol $fish^{-1}$)	28.4±2.5[a]	27.6±2.2[a]	95.1±6.7[b]
Apparent net Fe retention (%)	23.2	4.1	2.7
Proximate composition			
Moisture (%)	72.4±0.8	72.8±0.3	73.0±0.5
Protein (%)	56.0±0.5	54.7±2.0	55.0±1.5
Lipid (%)	18.4±0.9	17.5±0.9	19.4±0.1
Ash (%)	12.0±0.1	12.1±0.3	12.0±0.2

Values are mean ± S.E.M. At the end of the experiment, week 8, N=21 fish per treatment, except faecal Fe N=6, and whole body Fe content, N=9. Specific growth rate (SGR) and mean fed conversion ratio (FCR) were calculated from the mean mass gain/treatment between weeks 2 and 8. Whole body Fe contents are calculated from mean fish mass/treatment over the duration of the experiment and whole carcass Fe concentrations in Table 1. Whole body Fe content of initial fish was 17.7±0.9 μmol $fish^{-1}$. Apparent net Fe retention = (final carcass metal content – initial carcass metal content/metal consumed)×100%. Proximate composition is expressed as a percentage of dry matter (mean ± S.E.M., N=9 fish per treatment for moisture and N=3 tanks per treatment for pooled analysis of protein, lipid and ash. Initial fish moisture, protein, lipid and ash, respectively, were (% dry matter, mean ± S.E.M., N=6 fish per treatment for moisture and N=3 tanks per treatment for other parameters): 72.1±1.90, 57. 6±0.4, 18.1±0.4, 12.0±0.2%. No differences were observed in SGR, FCR, condition factor, HSI or proximate composition between initial and final fish (Student's t-test, P>0.05). Different letters indicate significant differences within rows (t-test, P<0.05).

N=9; low Fe, 0.320±0.031; normal Fe, 0.205±0.048; high Fe, 0.123±0.030). Similar statistically significant effects were noted in the liver at week 4 and the gill at week 6 (data not shown).

Discussion

Most previous studies on Fe uptake in salmonid fish have focused on minimum nutritional requirements for aquaculture (Watanabe et al., 1997; Andersen et al., 1996, 1998; Lorentzen and Maage, 1999). In this study we determined Fe status in several organs as well as the whole body, measured Fe handling in the serum and the ferrireductase activity that facilitates Fe^{2+} accumulation by tissues. This combination of measurements in fish fed either low- and high-Fe diets has, for the first time, enabled an overview of dietary Fe metabolism in rainbow trout. Overall, this study shows that rainbow trout have very different physiological responses to low and high dietary Fe intakes. In the latter, the gastrointestinal barrier does not prevent iron accumulation in the tissues, and fish show increased Fe binding to transferrin in the blood, and at the same time increased hepatic ferrireductase activity to facilitate rapid Fe storage in the liver. The strategy in Fe-deficient situations is very different, with fish up-regulating intestinal ferrireductase activity so that tissue Fe accumulation is maintained and thus body Fe status is relatively normal.

Normal iron metabolism in rainbow trout

In this study normal whole body Fe concentrations were around 0.2 μmol g^{-1} d.m. (11.2 μg g^{-1} d.m.; Table 1), which are comparable to the few other studies on rainbow trout, for example, using casein-based diets (0.9 μmol g^{-1} d.m.; Desjardins et al., 1987), and to those in Atlantic salmon fed commercial diets (*Salmo salar*, 0.44 μmol g^{-1} d.m., calculated from Andersen et al., 1996). Normal hepatic Fe levels in this study (1.3–2.0 μmol g^{-1} d.m.; Fig. 1) were also similar to previous reports for rainbow trout (e.g. 2.5 μmol g^{-1} d.m.; Lanno et al., 1985). Dietary Fe levels between 100 and 400 mg kg^{-1} (1.8–7.2 mmol kg^{-1} d.m. food) produced steady state hepatic Fe levels and blood haemoglobin concentrations in Atlantic salmon, suggesting the normal dietary Fe requirement for salmon is between 100 and 400 mg Fe kg^{-1} d.m. food (Andersen et al., 1996). Steady-state Fe concentrations in most organs (Fig. 1), normal haematology (Table 2) and hepatic morphology (Fig. 6), of control fish suggest the 'normal' control diet used in this study was within the physiologically normal range for rainbow trout. Unlike other studies on fish we also examined the body distribution of Fe (Table 1). The visceral organs of both initial and control fish support a small proportion (about 15%) of the whole body Fe load (as in mammals; Van Campen and Mitchell, 1965). This is also consistent with an early study in rainbow trout, where intraperitoneal injections of ^{59}Fe resulted in 14% of the radiotracer initially appearing in the liver and intestine (Walker and Fromm, 1976).

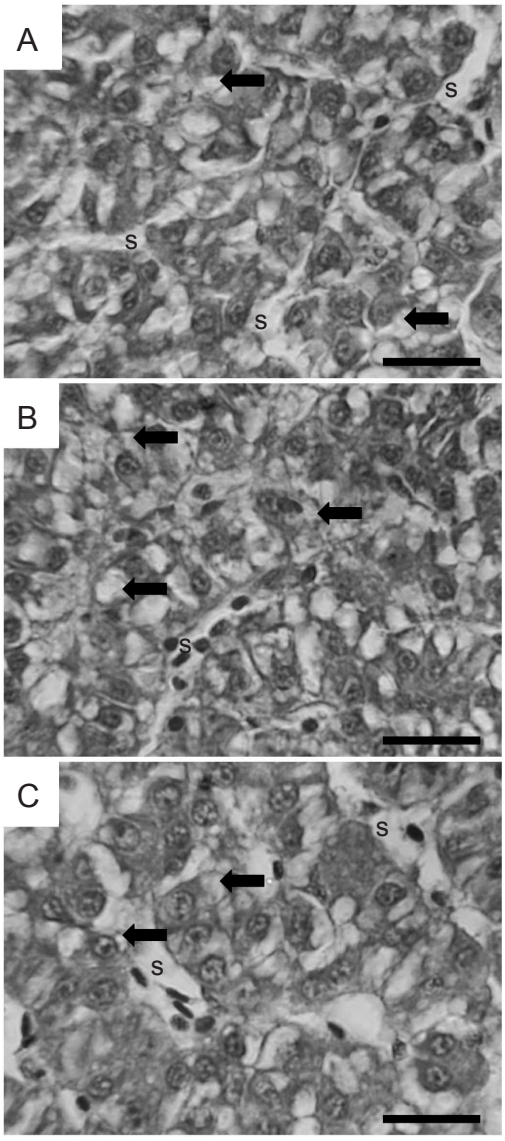

Fig. 6. Sections of liver (7 μm) stained with Mallory's trichrome from fish fed (A) low (B) normal- and (C) high-Fe diets for 8 weeks. Livers are healthy, but show some subtle changes in intracellular glycogen storage and sinusoid space between treatments. Fish fed on the low-Fe diet showed smaller hepatocytes, with less glycogen storage (arrows), and slightly more sinusoid space (S) than normal diet controls. Livers from fish fed the high-Fe diet had slightly more sinusoid space than controls, and had slightly larger (mild hypertrophy) hepatocytes that contained less glycogen than controls. Scale bars, 40 μm.

There are only a few measurements of transferrin concentration and percentage saturation of transferrin with Fe in healthy salmonid fish, and these are taken from wild populations or farmed fish (Ikeda et al., 1972; Hershberger and Pratschner, 1981). They suggest TIBC is around 65 μmol l^{-1} with about 23% saturation of transferrin with Fe (Hershberger and Pratschner, 1981), although this may vary with season and nutritional quality of the food (Ikeda et al., 1972). In trout on the normal diet, transferrin levels were around 85 μmol l^{-1}

(TIBC Fe equivalence; Fig. 2A), and between 10 and 21% of the transferrin was saturated with Fe when the total serum Fe concentration was about 10 μmol l^{-1}. The latter is consistent with the results of Walker and Fromm (1976) who recorded a similar value (total plasma Fe of 9.8 μmol l^{-1}) in controlled laboratory conditions. In humans, the normal clinical range for total serum Fe is 6–25 μmol l^{-1} (35–140 μg dl^{-1}; Sigma data sheet with Fe kit). Thus total Fe levels in trout serum are about the same as in humans. However, trout have less than half the circulating apotransferrin (iron-free transferrin) levels of humans (clinical range UIBS, 23.2–67.1 μmol l^{-1}), and a smaller proportion of the transferrin is saturated (trout 10–21%, Fig. 2C, and humans 13–45%). The TIBC of normal trout serum (80–90 μmol l^{-1}; Fig. 2A) was also higher than in humans (clinical range TIBC, 43–71 μmol l^{-1}). This may imply that trout normally use a smaller proportion of the circulating transferrin than humans, despite having higher circulating Fe levels than humans. Alternatively, other blood proteins (e.g. ferritin) in the blood of trout might contribute more to TIBC than in humans. Nonetheless, the rainbow trout model for Fe metabolism also seems much closer to that of humans than the rodent model. Rats have serum Fe concentration that are 2 fold or more higher than humans (about 35 μmol l^{-1} or 200 μg dl^{-1}; Horne et al., 1997), and normal transferrin saturation in rats is 50% or more (Horne et al., 1997).

Intestinal absorption of Fe in the mammalian involves the reduction of Fe^{3+} to Fe^{2+} in the brush border of the mucosal membrane, and this is achieved by a ferrireductase (Riedel et al., 1995; McKie et al., 2001). Ferrireductase activity has not been previously reported in fish, but its involvement in intestinal Fe absorption has been implicated by the relatively slow absorption of Fe^{3+} compared with Fe^{2+} by the gut of European flounder, *Platichthys flesus* (Bury et al., 2001). Ferrireductase activity in crude intestinal homogenates from normal trout was between 11.2 and 21.9 nmol mg^{-1} protein min^{-1} (Fig. 3B), and this is similar to that in homogenates of human intestinal cells (27 nmol mg^{-1} protein min^{-1}; Riedel et al., 1995). Furthermore, we also find ferrireductase activity in the gills and liver of normal trout (Fig. 3). The latter being increased when serum transferrin becomes saturated (see below and Fig. 4).

Iron metabolism in response to high dietary Fe

Increasing dietary Fe concentration from 175 to 1975 mg Fe kg^{-1} d.m. food caused Fe concentrations in the stomach, intestine, liver and serum to rise (Fig. 1). This was accompanied by a redistribution of whole body Fe, so that the proportion of whole body Fe held in the intestine and serum both increased 13 fold (Table 1). However on a body burden basis, the percentage of Fe in the liver did not increase suggesting that Fe storage in the liver keeps pace with Fe loading of the whole body. These changes in the distribution of whole body Fe occurred without alteration in haematological (Table 2) or nutritional performance (Table 3), and therefore suggest a physiological adjustment of Fe metabolism. The slight enlargement of liver cells of fish on the high Fe diet without pathology (Fig. 6) also suggests increased metabolic activity in the liver. This physiological response to elevated dietary Fe did not involve the prevention of Fe absorption at the intestine because tissue Fe levels generally increased (Fig. 1), and intestinal ferrireductase activity did not decline (remained normal; Fig. 3B). A lack of down regulation of ferrireductase in the presence of high dietary Fe is consistent with observations in mice, where levels of putative ferrireductase mRNA remain unaltered from control levels in mice fed Fe replete diets (McKie et al., 2001).

Instead, trout appear to regulate whole body Fe status during periods of high dietary Fe intake by modulating the labile Fe pool in the blood, which may subsequently enable transfer of Fe to the liver for storage. The total transferrin pool in the blood increased in response to elevation of serum Fe during high dietary Fe uptake (Figs 1D, 2). This resulted in a 4-fold increase in saturated transferrin (to 37% saturation; Fig. 2C). The presence of unsaturated transferrin (Fig. 2B) indicated ample spare capacity for Fe binding in the blood of fish fed the high-Fe diet, even though Fe accumulation by the intestine had increased. However, fish fed the high-Fe diet did increase Fe accumulation in the liver. Both hepatic Fe levels and ferrireductase activity were positively correlated with the percentage saturation of transferrin in the blood in fish fed normal and high-Fe diets (Fig. 4). Hepatic ferrireductase was also positively correlated with hepatic Fe concentrations (Fig. 3C). Together these observations suggest that hepatic ferrireductase enables Fe removal from the blood, and facilitates the accumulation of Fe^{2+} in the liver when serum Fe levels are normal or higher.

The precise mechanism of intracellular Fe handling in the liver of trout remains to be investigated, but the ubiquitous nature of ferritin in animal cells suggests that intracellular Fe will be stored as Fe^{3+} inside the core of cytoplasmic ferritin (Aisen et al., 2001). It could therefore be argued that any intracellular mechanism that promotes the oxidation of intracellular Fe^{2+} to Fe^{3+} would promote Fe storage by ferritin in the liver.

We also measured TBARS in the liver to monitor the general level of oxidation in the tissue. The TBARS values we report (<1 nmol mg $protein^{-1}$; Fig. 5) are at least an order of magnitude less than measurements made during sub-lethal toxicity in fish (as malondialdehyde equivalence; Baker et al., 1997; Baker et al., 1998), and therefore represent subtle physiological changes in oxidative status of the tissue during Fe metabolism. This notion is supported by the absence of oxidative damage in the livers of all fish (Fig. 6). Hepatic TBARS were positively correlated with Fe concentration in the liver at the end of the experiment (insert, Fig. 5C). However this is not evidence for spontaneous auto-oxidation of Fe^{2+} to Fe^{3+} to promote hepatic Fe storage by ferritin, because hepatic Fe levels increased several weeks prior to changes in TBARS (Figs 1 and 5). Instead the slight elevation of hepatic TBARS is a consequence of excess Fe in the liver. Normal HSI

(Table 3), hepatic ferrireductase (Fig. 3) and gross histology (Fig. 6) also argues against spontaneous oxidation of Fe^{2+} to Fe^{3+} in the liver. Similar arguments apply to the intestine where a small but persistent elevation of intestinal TBARS were positively correlated with increasing Fe levels in the gut (Fig. 5B), but did not undermine nutritional performance (Table 3).

Several authors argue against the involvement of the gills in Fe uptake (see Bury et al., 2003), and evidence here suggests the gills do not have a primary role in Fe storage and excretion during high dietary-Fe intake. The absence of clear changes in branchial Fe levels, ferrireductase or TBARS (Figs 1, 3, 5) implies that the gill were not involved in the regulation of excess Fe absorption. Indeed, the proportion of the body burden held by the gills decreased in response to the high-Fe diet (Table 1).

Iron metabolism in response to low dietary Fe

The low-Fe diet generally had no effect on tissue Fe levels (Fig. 1) or Fe distribution (Table 1) compared with the normal diet, except for a 50% reduction in Fe distribution to the stomach. However, there were some changes in Fe handling in the serum. The percentage saturation of transferrin in the blood of fish on the low-Fe diet was less than controls by the end of the experiment (Fig. 2C). The total Fe content in the serum of fish fed the low-Fe diet was marginally (and consistently) lower than that in fish fed the normal diet (Fig. 1). Similar observations were made by Walker and Fromm (1976), when, despite repeated bleeding to reduce whole body iron levels over 30 days, the serum of Fe-deficient fish remained only marginally lower than controls. In the present study, fish fed the low-Fe diet also showed increased intestinal ferrireductase activity over both controls and fish on the high-Fe diet (Fig. 3B). Together these data suggest that trout maintained tissue Fe status by increasing Fe acquisition, probably facilitated by the intestinal ferrireductase (as in mice, McKie et al., 2001). This notion would also explain why intestinal ferrireductase was inversely correlated with intestinal Fe concentration in fish on the low and high-Fe diets (inset, Fig. 3B). The reduction in saturation of transferrin in fish fed the low-Fe diet compared with controls (Fig. 2C) might also suggest that the labile pool of Fe in the serum (although small) was also used to partly maintain tissues Fe levels.

However, we may have also revealed different functional isoforms of the intestinal ferrireductase in trout. Data from control fish were clustered away from the regression line for the high- and low-Fe fish (inset, Fig. 3B). This suggests that the inducible ferrireductase in the intestine may not be the same as the apparently normal isoform of the enzyme in control fish, or alternatively the control fish normally use an Fe absorption pathway that does not require ferrireductase. The former seems more likely given the expression of three transcripts of ferrireductase-related mRNAs in the duodenum of mice (McKie et al., 2001) and the presence of NADH-dependent and independent ferrireductase in human intestinal cells (63% NADH dependent; Riedel et al., 1995). We also incidentally noted an NADH-independent component in trout intestine (data not shown). A 5-fold higher net Fe retention in fish fed the low-Fe diet than in controls (Table 3) also supports the notion of improved Fe absorption across the gut. The significance of slight reductions in glycogen stores and increased sinusoid space in the livers of fish on the low-Fe diet compared with controls is unclear given the normal nutritional performance of all fish (Table 3). There were no obvious trends in branchial Fe levels, ferrireductase, or TBARS in the gills of fish fed low-Fe diets compared with controls (Figs 1, 3, 5), indicating that fish were not making good dietary deficiency by Fe absorption at the gills (in agreement with Bury et al., 2003).

Trace element interactions with Fe metabolism

There have been a number of nutritional studies in which interactions between Fe, Zn, Cu and Mn metabolism have been suspected (e.g. Winzerling and Law, 1997; Knox et al., 1984; Lanno et al., 1985; Andersen et al., 1996). Elevation of dietary Cu intake causes increased Fe accumulation in the liver of trout (Lanno et al., 1985) without appreciable changes in total plasma Fe (Knox et al., 1984), and this might be explained by the presence of an intracellular ceruloplasmin-like ferroxidase, which enables Fe loading into hepatic ferritin (Reilly and Aust, 1998). Low dietary-Fe also alters Cu metabolism. In this study we found that intestinal Cu concentrations increased by 174% by the end of the experiment. This may reflect the ability of the intestinal Fe carrier, DMT1, to import Cu in to the gut enterocytes in absence of Fe (Gunshin et al., 1997). Similarly, DMT1 will also import Mn in the absence of Fe (Gunshin et al., 1997) and might explain the observed increases in intestinal Mn (increased by 65%, week 8) in fish fed the low-Fe diet. Andersen et al. (1996) also noted an inverse relationship between dietary Fe level and tissue Mn. This relationship can be partly explained by the characteristics of DMT1, but transferrin also binds Mn^{2+} (Nelson, 1999), and increased UIBC in fish on the high-Fe diet (Fig. 2) may facilitate Mn accumulation.

In conclusion, rainbow trout closely regulate Fe status by increasing Fe-binding to transferrin in the blood and promoting hepatic Fe accumulation, probably facilitated by hepatic ferrireductase activity when dietary Fe intake is high. Trout resist iron depletion when dietary Fe intake is low by up regulating intestinal ferrireductase which probably results in improved Fe accumulation in the intestine. The characteristics of Fe storage in the blood of trout and patterns of tissue Fe accumulation, coupled with the potential utility of rainbow trout for molecular studies of Fe metabolism (Bury et al., 2003), suggest the rainbow trout is a good model for future studies on vertebrate Fe metabolism.

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